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## Seven New Records of *Penicillium* Species Belonging to Section *Lanata-Divaricata* in Korea

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### ABSTRACT

*Penicillium* species are known to be ubiquitous environmental saprophytes. In the survey of diversity of genus *Penicillium*, seven new records of *Penicillium* species belonging to section *Lanata-Divaricata* were isolated from freshwater and soil samples collected from different locations in Korea. Based on morphological characteristics and multilocus phylogenetic analysis of the rDNA internal transcribed spacer region (ITS),  $\beta$ -tubulin (*BenA*), and calmodulin (*CaM*) genes, the isolated strains were identified as *P. annulatum*, *P. camponotum*, *P. echinulonalgiovense*, *P. globosum*, *P. limosum*, *P. onobense*, and *P. yunnanense*, respectively. This study presents detailed phylogenetic analyses and morphological descriptions of these species that contribute to section *Lanata-Divaricata* in Korea.

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Freshwater; soil; section *Lanata-Divaricata*; morphology; phylogeny

## 1. Introduction

The genus *Penicillium* established by Link et al. in 1809 [1], has a worldwide distribution, isolated from diverse substrates, including air, soil, freshwater, as endophytes, insect specimens, indoor environments, and food products [2,3]. Using phylogenetic approach, often supported by phenotypic, physiologic and/or extrolite data, members of this genus is divided into two subgenera, 32 sections and 89 series [2]. Members of this genus are economically important as it produce antibiotics, enzymes, organic acids, alcohols and pharmaceuticals [4]. Whereas, some of them cause food spoilage, produce mycotoxins, and cause human and animal diseases [5]. Currently, the genus contains 483 accepted species [2]. In Korea, more than 100 *Penicillium* species have been reported [6–12].

The *Penicillium* section *Lanata-Divaricata* was established by Thom et al. in 1930 [13] for species with biverticillate conidiophores that usually contain an elongation of the conidiophore's main axis and metulae that diverge from axis to form an asymmetrical verticil. Thus, resulting in conidiophores to be interpreted as monoverticillate, although they are in most cases divergently branched biverticillate conidiophores (also termed divaricate). This group of species mainly isolated from soil, some found from air, and protea repens infructescence [3,14–16]. This section is species-rich, with 56 species are accepted until 2016 [14–19]. But the list is rapidly increasing

with many new *Penicillium* species recently described from all over the world and added to section *Lanata-Divaricata*. Up to date, 76 species of *Penicillium* sect. *Lanata-Divaricata* have been accepted [2]. Recently, *P. soli*, *P. melanosporum*, *P. siccitolerans* and *P. michoacanense*, were discovered from phosphate solubilizing soil in China and soil samples as xerophilic in Mexico and Spain [20,21].

To our knowledge, only 16 species of *Penicillium* section *Lanata-Divaricata* have been reported in Korea until now [6,10,11]. Thus, the aim of this study was to isolate, identify and describe the previously unrecorded seven isolates found in soil and freshwater samples collected from different locations in Korea, *P. annulatum*, *P. camponotum*, *P. echinulonalgiovense*, *P. globosum*, *P. limosum*, *P. onobense*, and *P. yunnanense* based on multi-loci phylogenetic analysis of ITS, *BenA* and *CaM*, and morphological data.

## 2. Materials and methods

### 2.1. Sampling and isolation

Details of freshwater and soil samples collected from different locations in South Korea are shown in Table 1. Serial dilution plating method was used to isolate fungal strains; 1 g of soil or 1 mL of freshwater sample was added to 9 mL sterile distilled water. Approximately,  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ , and  $10^{-4}$  dilutions were plated onto potato dextrose agar (PDA) (Difco™, Sparks, MD, USA) and malt extract agar

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**Table 1.** Information of *Penicillium* isolates used in this study.

Species	Strain	Culture Collection	Source	Location
<i>Penicillium annulatum</i>	CNUFC ULD4-13	IMYKFGC000000043	Rhizosphere soil	Seonginbong Peak, Ulleung Island, Korea (37°30'04" N 130°51'23"E)
<i>Penicillium camponotum</i>	CNUFC ULD4-3	QWJQFGC000000303	Rhizosphere soil	Seonginbong Peak, Ulleung Island, Korea (37°30'04" N 130°51'23"E)
<i>Penicillium echinulonalgiovense</i>	CNUFC MDW-11	QWJQFGC000000469	Freshwater	Mt. Mudeung, Wonhyo valley, Gwangju, Korea (35°08'21" N 126°59'33"E)
<i>Penicillium globosum</i>	CNUFC WG2-7	NNIBRFG25626	Freshwater	Jeongdori Gugyedeung, Wando, Korea (34°19'1.20" N 126°45'0.00" E)
<i>Penicillium limosum</i>	CNUFC DMS3-17	QWJQFGC000000008	Damp soil	Sandeok-ri, Goseomyeon, Damyang, Korea (35°19'16.1" N 126°59'17.401" E)
<i>Penicillium onobense</i>	CNUFC WG2-1	NNIBRFG9318	Freshwater	Jeongdori Gugyedeung, Wando, South Korea (34°19'1.20" N 126°45'0.00" E)
<i>Penicillium yunnanense</i>	CNUFC WNP1	NNIBRFG25628	Freshwater	Doam-gil, Wando, Korea (34°19'1.20" N 126°45'0.00" E)

(MEA) (Difco™) containing 50 ppm streptomycin solution. Plates were incubated at 25 °C for 7–10 days. Then, colonies were transferred to new PDA plates and incubated for 7 days at 25 °C.

For stock storage, pure isolates were maintained in 20% glycerol at –80 °C and in PDA slant tubes in the Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, Korea. The isolated strains were also deposited in the Collection of National Institute of Biological Resources (NIBR), Incheon, and Culture Collection of the Nakdonggang National Institute of Biological Resources (NNIBR), Sangju, Korea as shown in Table 1.

## 2.2. DNA extraction and PCR sequencing

Total genomic DNA was extracted directly from the mycelia using Solg TM Genomic DNA Prep Kit (Solgent Co. Ltd., Daejeon, Korea). The ITS rDNA region was amplified with primer pairs ITS1/ITS4 or ITS5/LR5 [22], *BenA* gene with T10/Bt2b [23] or T1/Bt2b [23,24], and *CaM* gene were amplified with CF1/CF4 [25], respectively. The PCR cycling programs used for amplification follows: initial denaturation at 94 °C for 4 min, followed by denaturing at 94 °C for 1 min, then annealing (in case of ITS rDNA and *BenA*) at 56 °C for 30 s (in case of *CaM*, annealing temperature at 55 °C for 50 s), extension for 2 min at 72 °C, and a final 10 min elongation step at 72 °C which was followed by cooling at 4 °C for 30 cycles. PCR products were visualized in 1% (w/v) agarose gel electrophoresis. The PCR products were purified with the Accuprep PCR Purification Kit (Bioneer Corp., Daejeon, Korea). PCR products were sequenced using the same PCR primers on an ABI PRISM3730XL Genetic Analyzer (Applied Biosystems, CA, USA) at Macrogen (Daejeon, Korea).

## 2.3. Phylogenetic analysis

All sequence data used in this study were obtained from GenBank. Sequences were aligned with

Clustal\_X version 2.1 [26] and were edited manually with Bioedit version 7.2.6.0 [27]. Maximum likelihood (ML) phylogenies were assessed using MEGA 7 software [28] and the Kimura 2-parameter model. The p-distance substitution model with 1,000 bootstrap replications was used for the assessment of the reliability of internal branches. *Penicillium glabrum* CBS 125543 (T) was used as outgroup. The sequences of the isolates in this study were deposited in the database under the accession numbers shown in Table 2.

## 2.4. Morphological studies

For characterization, the respective strains were inoculated into three points, namely Czapek yeast autolysate agar (CYA), Blakeslee's malt extract agar (MEA), and yeast extract sucrose agar (YES), and incubated at 25 °C for 7 days [29]. For morphological observations, fragments of mycelia were removed from the cultures and placed on microscope slides with lactic acid (60%). An Olympus BX51 microscope (Olympus, Tokyo, Japan) was used to capture digital images.

## 3. Results

### 3.1. Phylogenetic analysis

A BLASTn search on the ITS, *BenA*, and *CaM* regions of the isolates were obtained. ITS regions of CNUFC ULD4-13, CNUFC ULD4-3, CNUFC MDW-11, CNUFC WG2-7, CNUFC DMS3-17, CNUFC WG2-1, and CNUFC WNP1 showed similarities of 99.8% (498/499 bp), 99.8% (485/486 bp), 99.8% (501/502 bp), 99.8% (488/489 bp), 100% (533/533 bp), 100% (533/533 bp), and 99.8% (481/482 bp) with *P. annulatum* (NR\_138303), *P. camponotum* (NR\_158823), *P. echinulonalgiovense* (GU981587), *P. globosum* (KY495018), *P. limosum* (NR\_111496), *P. onobense* (NR\_111497), and *P. yunnanense* (KY494989), respectively. In a BLASTn search with *BenA* sequence, the isolate CNUFC ULD4-13,

**Table 2.** GenBank accession numbers for fungal strains used in this study.

Species	Strain	GenBank Accession no.		
		ITS	<i>BenA</i>	<i>CaM</i>
<i>P. alagoense</i>	URM 93058 (T)	MK804503	MK802333	MK802336
<i>P. annulatum</i>	CBS 135126 (T)	JX091426	JX091514	JX141545
<b><i>P. annulatum</i></b>	<b>CNUFC ULD4-13</b>	<b>MZ234244</b>	<b>MZ284832</b>	<b>MZ284839</b>
<i>P. araracuarensis</i>	CBS 113149 (T)	GU981597	GU981642	KF296373
<i>P. bissettii</i>	CBS 140972 (T)	KT887845	KT887806	KT887767
<i>P. brasiliense</i>	CBS 253.55 (T)	GU981577	GU981629	MN969239
<i>P. brefeldianum</i>	CBS 235.81 (T)	AF033435	GU981623	AB667857
<i>P. camponotum</i>	CBS 140982 (T)	KT887855	KT887816	KT887777
<b><i>P. camponotum</i></b>	<b>CNUFC ULD4-3</b>	<b>MZ234245</b>	<b>MZ284833</b>	<b>MZ284840</b>
<i>P. caperatum</i>	CBS 443.75 (T)	KC411761	GU981660	KF296392
<i>P. cataractum</i>	CBS 140974 (T)	KT887847	KT887808	KT887769
<i>P. cluniae</i>	CBS 326.89 (T)	KF296406	KF296471	KF296402
<i>P. coeruleum</i>	CBS 141.45 (T)	GU981606	GU981655	KF296393
<i>P. cremeogriseum</i>	CBS 223.66 (T)	GU981586	GU981624	KF296403
<i>P. curticaule</i>	CBS 135127 (T)	FJ231021	JX091526	JX141536
<i>P. echinulonalgioense</i>	CBS 328.59 (T)	GU981587	GU981631	KX961269
<b><i>P. echinulonalgioense</i></b>	<b>CNUFC MDW-11</b>	<b>MZ234246</b>	<b>MZ284834</b>	<b>MZ284841</b>
<i>P. ehrlichii</i>	CBS 324.88 (T)	AF033432	GU981652	KF296395
<i>P. elleniae</i>	CBS 118135 (T)	GU981612	GU981663	MN969254
<i>P. excelsum</i>	DTO 357-D7 (T)	KR815341	KP691061	KR815342
<i>P. fructuariae-cellae</i>	CBS 145110 (T)	MK039434	KU554679	MK045337
<i>P. glaucoroseum</i>	CBS 138908 (T)	MN431390	MN969383	MN969257
<i>P. globosum</i>	CBS 144639 (T)	KY495014	KY495123	KY494954
<b><i>P. globosum</i></b>	<b>CNUFC WG2-7</b>	<b>MZ234247</b>	<b>MZ284835</b>	<b>MZ284842</b>
<i>P. griseoflavum</i>	CGMCC 3.18799 (T)	KY495011	KY495120	KY494951
<i>P. guangxiense</i>	CBS 144526 (T)	KY494986	KY495095	MN969332
<i>P. hainanense</i>	CBS 144527 (T)	KY495009	KY495118	KY494949
<i>P. infrabuccalum</i>	CBS 140983 (T)	KT887856	KT887817	KT887778
<i>P. janthinellum</i>	CBS 340.48 (T)	GU981585	GU981625	MN969268
<i>P. javanicum</i>	CBS 341.48 (T)	GU981613	GU981657	GU981613
<i>P. koreense</i>	KACC 47721 (T)	KJ801939	KM000846	N/A
<i>P. laevigatum</i>	CGMCC 3.18801 (T)	KY495015	KY495124	KY494955
<i>P. levitum</i>	CBS 345.48 (T)	GU981607	GU981654	KF296394
<i>P. limosum</i>	CBS 339.97 (T)	GU981568	GU981621	KF296398
<b><i>P. limosum</i></b>	<b>CNUFC DMS3-17</b>	<b>MZ234248</b>	<b>MZ284836</b>	<b>MZ284843</b>
<i>P. lineolatum</i>	CBS 188.77 (T)	GU981579	GU981620	MN969272
<i>P. ludwigii</i>	CBS 417.68 (T)	KF296409	KF296468	MN969273
<i>P. malacosphaerulium</i>	CBS 135120 (T)	FJ231026	JX091524	JX141542
<i>P. mariae-crucis</i>	CBS 271.83 (T)	GU981593	GU981630	KF296374
<i>P. meloforme</i>	CBS 445.75 (T)	KC411762	GU981656	KF296396
<i>P. ochrochloron</i>	CBS 357.48 (T)	GU981604	GU981672	KF296378
<i>P. onobense</i>	CBS 174.81 (T)	GU981575	GU981627	KF296371
<b><i>P. onobense</i></b>	<b>CNUFC WG2-1</b>	<b>MZ234249</b>	<b>MZ284837</b>	<b>MZ284844</b>
<i>P. ortum</i>	CBS 135669 (T)	JX091427	JX091520	JX141551
<i>P. panisanguineum</i>	CBS 140989 (T)	KT887862	KT887823	KT887784
<i>P. paraherquei</i>	CBS 338.59 (T)	AF178511	KF296465	KF296372
<i>P. pedernalense</i>	CBS 140770 (T)	KU255398	KU255396	N/A
<i>P. piscarium</i>	CBS 362.48 (T)	GU981600	GU981668	KF296379
<i>P. pulvillorum</i>	CBS 280.39 (T)	AF178517	GU981670	KF296377
<i>P. raperi</i>	CBS 281.58 (T)	AF033433	GU981622	MN969291
<i>P. reticulisporum</i>	NRRL 3447 (T)	AF033437	GU981665	KF296391
<i>P. rolfsii</i>	CBS 368.48 (T)	JN617705	GU981667	KF296375
<i>P. rubriannulatum</i>	CBS 144641 (T)	KY495029	KY495138	KY494969
<i>P. setosum</i>	CBS 144865 (T)	KT852579	MF184995	MF105905
<i>P. simplicissimum</i>	CBS 372.48 (T)	GU981588	GU981632	KF296368
<i>P. skrjabinii</i>	CBS 439.75 (T)	GU981576	GU981626	KF296370
<i>P. soliforme</i>	CBS 144482 (T)	KY495038	KY495147	MN969337
<i>P. spinuliferum</i>	CBS 144483 (T)	KY495040	KY495149	MN969338
<i>P. subrubescens</i>	DTO 188-D6 (T)	KC346350	KC346327	KC346330
<i>P. svalbardense</i>	CBS 122416 (T)	GU981603	KC346325	KC346338
<i>P. tanzanicum</i>	CBS 140968 (T)	KT887841	KT887802	KT887763
<i>P. terrarumae</i>	CBS 131811 (T)	MN431397	KX650295	MN969323
<i>P. vasconiae</i>	CBS 339.79 (T)	GU981599	GU981653	KF296386
<i>P. wotroi</i>	CBS 118171 (T)	GU981591	GU981637	KF296369
<i>P. yunnanense</i>	CBS 144485 (T)	KY494990	KY495099	KY494930
<b><i>P. yunnanense</i></b>	<b>CNUFC WNP1</b>	<b>MZ234250</b>	<b>MZ284838</b>	<b>MZ284845</b>

Bold letters indicate isolates and accession numbers determined in our study.

CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CNUFC: Chonnam National University Fungal Collection (Gwangju, South Korea); DTO: Working collection of the Applied and Industrial Mycology department housed at the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; 7); KACC: Korean Agricultural Culture Collection, National Institute of Agricultural Biotechnology, Rural Development Administration (Suwon, South Korea); URM: Culture collection (WCDM 604) hosted at the Departamento de Micologia of the Universidade Federal de Pernambuco, Recife, Brazil. T: ex-type strain; N/A: Not available.

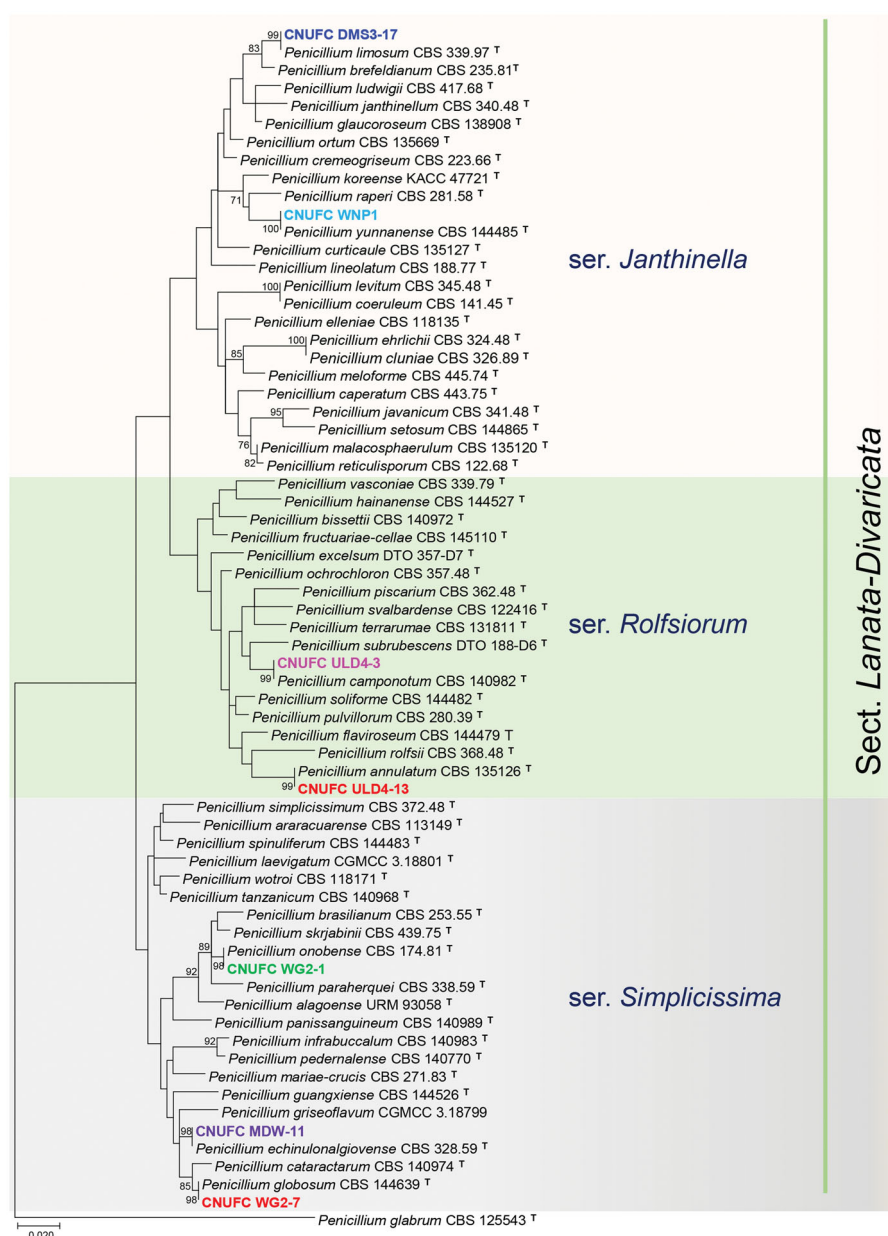
CNUFC ULD4-3, CNUFC MDW-11, CNUFC WG2-7, CNUFC DMS3-17, CNUFC WG2-1, and CNUFC WNP1 showed similarity 99.6% (470/472 bp), 99.17% (479/483 bp), 98.80% (411/416 bp), 100% (416/416 bp), 99.79% (466/467 bp), 100% (480/480 bp), and 99.76% (411/412 bp) with sequences of *P. annulatum* CV0037 (JX091514), *P. camponotum* (KT887819), *Penicillium* sp. YD-2017a (KY495150), *P. globosum* (KY495127), *P. limosum* (GU981621), *P. onobense* (GU981627), and *P. yunnanense* (KY495099), respectively. Similarly, *CaM* sequence of CNUFC ULD4-13, CNUFC ULD4-3, CNUFC MDW-11, CNUFC WG2-7, CNUFC DMS3-17, CNUFC WG2-1, and CNUFC WNP1 showed similarities of 98.53% (536/544 bp), 98.64% (436/442 bp), 98.51% (398/404 bp), 99.5% (402/404 bp), 100% (407/407 bp), 99.75% (405/406 bp), and 99.75% (405/

406 bp) with *P. annulatum* (JX141547), *P. camponotum* (KT887781), *Penicillium* sp. YD-2017a (KY494981), *P. globosum* (KY494958), *P. limosum* (KF296398), *P. onobense* (KF296371), and *P. yunnanense* (KY494930), respectively. Phylogenetic tree based on the combined sequence data of the three loci, ITS, *BenA*, and *CaM* revealed that the seven isolated strains were identical to *P. annulatum*, *P. camponotum*, *P. echinulonalgiovense*, *P. globosum*, *P. onobense*, *P. limosum*, and *P. yunnanense* (Figure 1).

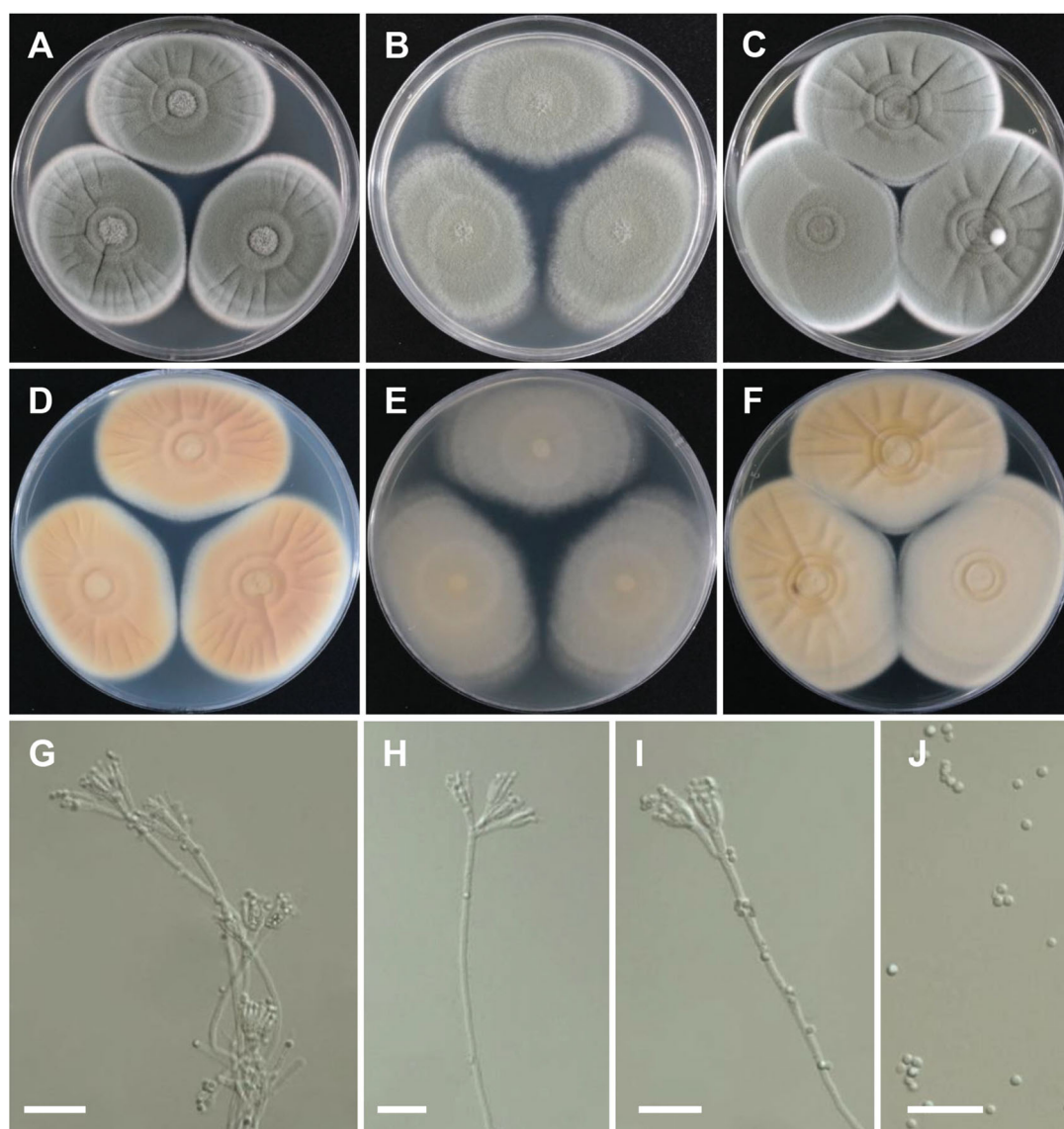
### 3.2. Taxonomy

#### 3.2.1. Taxonomy of CNUFC ULD4-13

*Penicillium annulatum* Visagie & K. Jacobs, Mycological Progress 14 (10/96): 14 (2015) (Figure 2).



**Figure 1.** Phylogenetic tree of *Penicillium annulatum* CNUFC ULD4-13, *P. camponotum* CNUFC ULD4-3, *P. echinulonalgiovense* CNUFC MDW-11, *P. globosum* CNUFC WG2-7, *P. limosum* CNUFC DMS3-17, *P. onobense* CNUFC WG2-1, and *P. yunnanense* CNUFC WNP1 and related species belonging to section *Lanata-Divariata* based on maximum likelihood analysis of the combined ITS, *BenA*, and *CaM* sequences. Numbers at the nodes indicate the bootstrap values (>70%) from 1,000 replicates. The bar indicates the number of substitutions per nucleotide. The study isolates are shown in bold and different colors.



**Figure 2.** Morphology of *Penicillium annulatum*. (A, D) Colonies on Czapek yeast autolysate agar (CYA); (B,E) Colonies on Blakeslee's malt extract agar (MEA); (C,F) Colonies on yeast malt extract agar (YES). (A–C: obverse view, D–F: reverse view). (G–I) Conidiophores; (J) Conidia (scale bars: G–J = 20 µm).

Colony characteristics: CYA 25 °C, 7 days: colonies were moderately deep, radially and concentrically sulcate, with ring-like appearance because of sporulating and nonsporulating areas, with low margins, light-green to white mycelia, floccose texture, sparse to moderately dense sporulation, no soluble pigment, reverse light to grayish orange, and reached 40–46 mm in diameter. MEA 25 °C, 7 days: colonies were low to moderately deep, plane, low, irregular margins, light-green to white mycelia, floccose texture, moderately dense to dense sporulation, no exudate, no soluble pigment, reverse dull-green to greenish white, and reached 42–47 mm in diameter. YES 25 °C, 7 days: colonies were radially sulcate, with ring-like appearance because of sporulating and nonsporulating areas but less than those on CYA, low, narrow margins, white mycelia, floccose texture, sparse to moderate sporulation, no

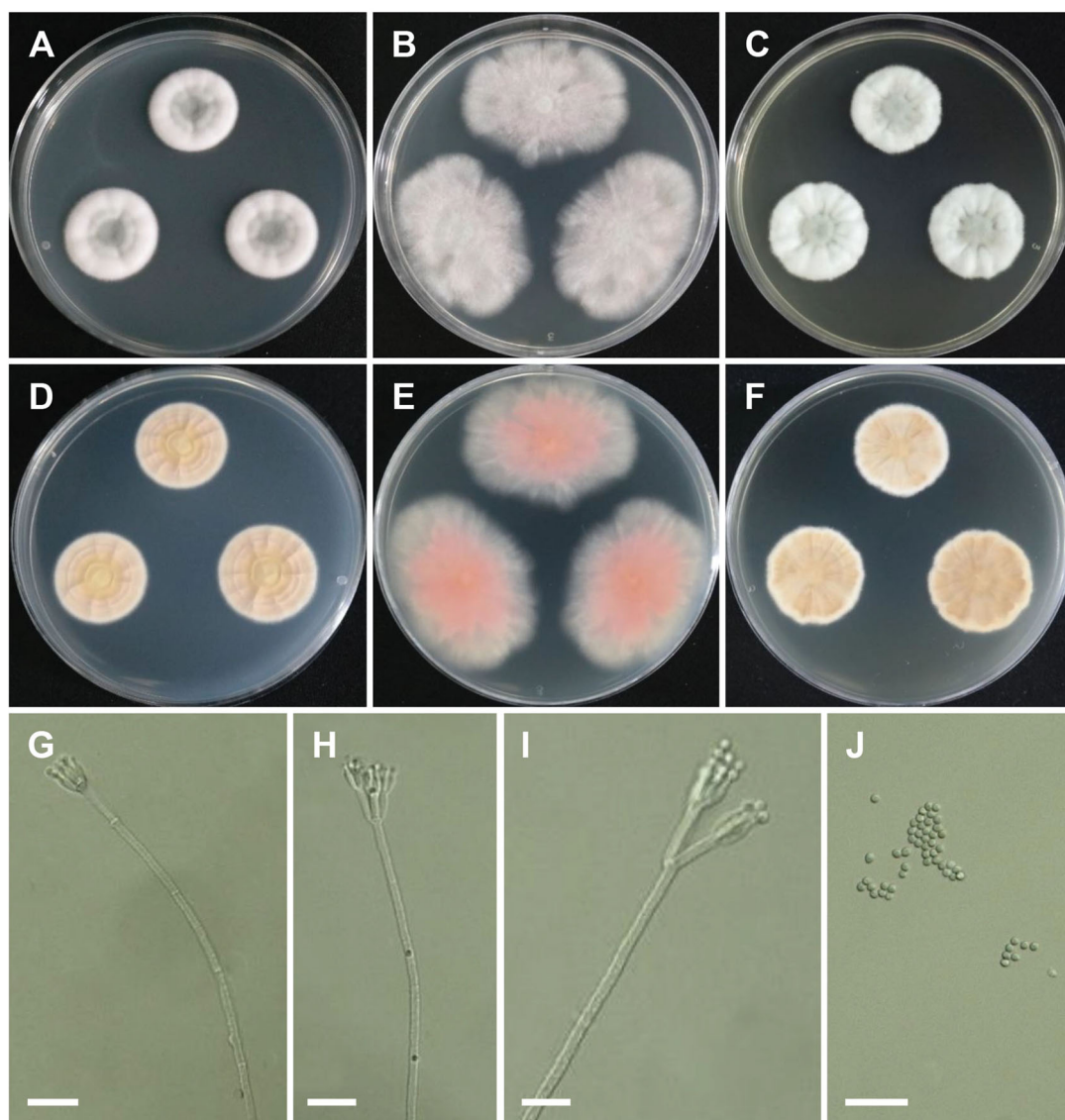
exudate and soluble pigment, reverse dull-yellow to yellowish white, and reached 41–49 mm in diameter.

Micromorphology: Conidiophores were bi- and terverticillate,  $165\text{--}720 \times 3\text{--}4.5\text{ }\mu\text{m}$ . Metulae were appressed to divergent,  $7.8\text{--}20 \times 2.5\text{--}4.5\text{ }\mu\text{m}$ . Phialides were ampulliform 3–5 per metula,  $6.2\text{--}8 \times 2\text{--}3.5\text{ }\mu\text{m}$ . Conidia were globose to subglobose,  $2.5\text{--}3 \times 2\text{--}3\text{ }\mu\text{m}$ .

### 3.2.2. Taxonomy of CNUFC ULD4-3

*Penicillium camponotum* Visagie, David Clark, & Seifert, Persoonia 36: 271 (2016) (Figure 3).

Colony characteristics: CYA 25 °C, 7 days: colonies were moderately deep, lightly radially sulcate, narrow, entire margins, white mycelia, floccose texture, sparse to absent sporulation, no soluble pigments, reverse yellowish white to pale-yellow, and reached 20–22 mm in diameter. MEA 25 °C, 7 days:



**Figure 3.** Morphology of *Penicillium camponotum*. (A,D) Colonies on Czapek yeast autolysate agar (CYA); (B,E) Colonies on Blakeslee's malt extract agar (MEA); (C,F) Colonies on yeast malt extract agar (YES). (A–C: obverse view, D–F: reverse view). (G–I) Conidiophores; (J) Conidia (scale bars: G–J = 20 µm).

colonies were plane, entire margins, white mycelia, floccose texture; moderately dense sporulation, no soluble pigments, no exudates, reverse pinkish gray, and reached 21–26 mm in diameter. YES 25 °C, 7 days: colonies were moderately deep, radially, and concentrically sulcate, with low, wide, entire margins, white mycelia, floccose texture, moderately dense sporulation, no soluble pigments, reverse light-brown, and reached 21–23 mm in diameter.

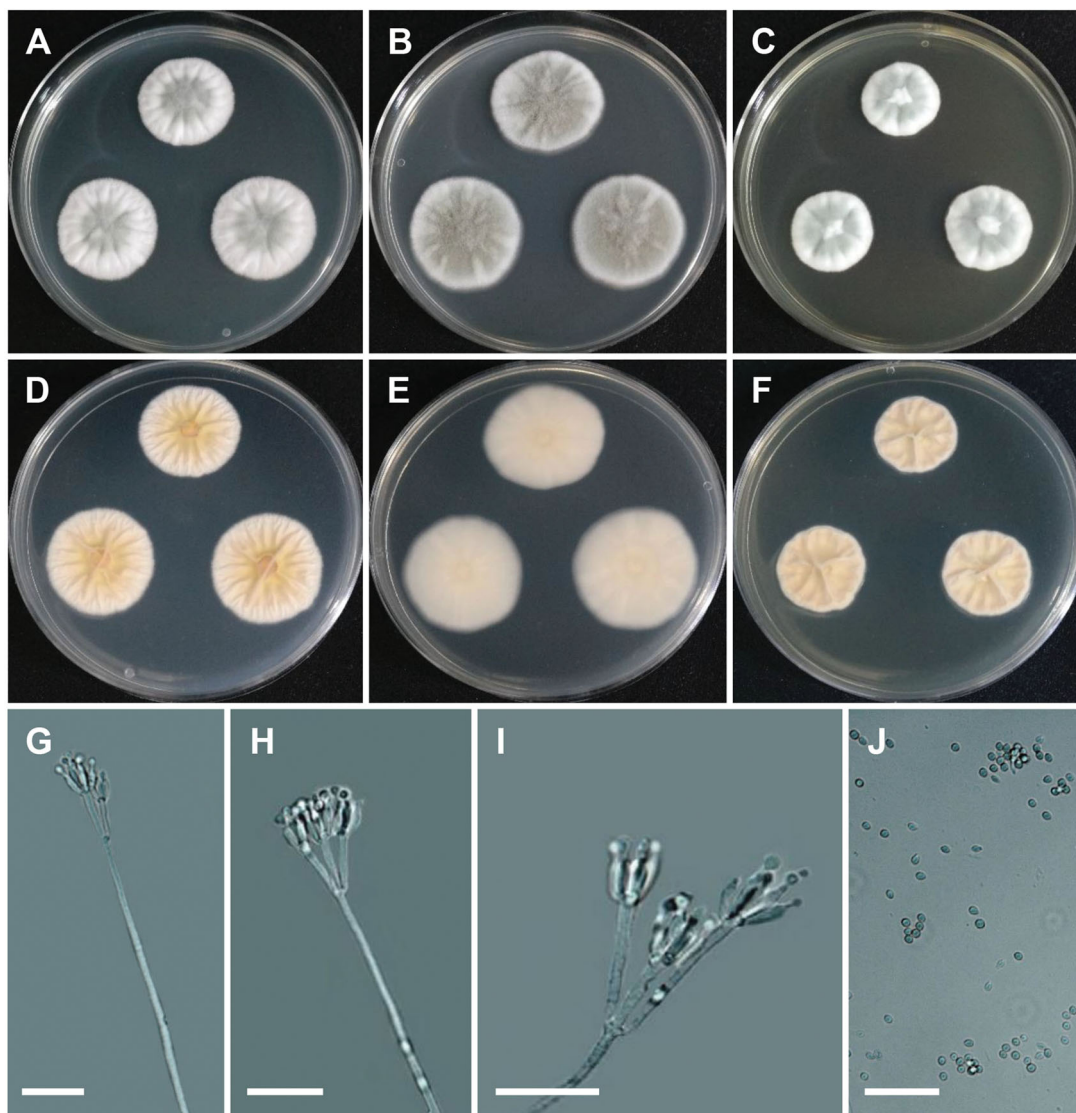
**Micromorphology:** Conidiophores were biverticillate,  $212\text{--}610 \times 2.5\text{--}3.5\text{ }\mu\text{m}$ . Metulae were divergent,  $12.2\text{--}25.6 \times 2.5\text{--}4\text{ }\mu\text{m}$ . Phialides were ampulliform, 3–6 per metula,  $8.2\text{--}11.4 \times 2.5\text{--}3.5\text{ }\mu\text{m}$ . Conidia were globose,  $2.2\text{--}3.5 \times 2.1\text{--}3.4\text{ }\mu\text{m}$ .

### 3.2.3. Taxonomy of CNUFC MDW-11

*Penicillium echinulonalgioense* S. Abe ex Houbraken & R.N. Barbosa, Antonie van Leeuwenhoek 111 (10): 1895 (2018) (Figure 4).

**Colony characteristics:** CYA 25 °C, 7 days: colonies were moderately deep and lightly radially sulcate, with low, narrow, entire margins, white mycelia, floccose texture, sparse to absent sporulation, no soluble pigments, reverse yellowish white to pale-yellow, and reached 18–22 mm in diameter. MEA 25 °C, 7 days: colonies were low to moderately deep and plane, with low, irregular margins, light-green to white mycelia, floccose texture, moderately dense to dense sporulation, no exudate, no soluble pigment, reverse dull-green to greenish white, and reached 20–23 mm in diameter. YES 25 °C, 7 days: colonies were low to moderately deep, sunken at center, radially and concentrically sulcate, with low, narrow, entire margins, white mycelia, floccose texture, sparse to moderately dense sporulation, no exudate and soluble pigment, reverse dull-green, and reached 17–20 mm in diameter.

**Micromorphology:** Conidiophores were biverticillate,  $56\text{--}210 \times 2.2\text{--}3.0\text{ }\mu\text{m}$ . Metulae were divergent,



**Figure 4.** Morphology of *Penicillium echinulonalgiense*. (A,D) Colonies on Czapek yeast autolysate agar (CYA); (B,E) Colonies on Blakeslee's malt extract agar (MEA); (C,F) Colonies on yeast malt extract agar (YES). (A–C: obverse view, D–F: reverse view). (G–I) Conidiophores; (J) Conidia (scale bars: G–J = 20 µm).

10.7–18.8 × 2.2–3.1 µm. Phialides were ampulliform, 2–6 per metula, 7.1–10.9 × 2.5–3.0 µm. Conidia were echinulate, globose to subglobose 2.5–3.9 × 2.3–3.7 µm.

### 3.2.4. Taxonomy of CNUFC WG2-7

*Penicillium globosum* L. Cai, Houbaken, & X.Z. Jiang, Cladistics 35 (5): 529 (2018) (Figure 5).

Colony characteristics: CYA 25°C, 7 days: colonies were sunken in center, radially and concentrically sulcate, low, narrow, entire margins, blueish white mycelia, floccose texture, sparse to moderately dense sporulation, no soluble pigment, reverse pale orange to apricot, and reached 20–22 mm in diameter. MEA 25°C, 7 days: colonies were low, irregular margins, white mycelia, floccose texture, sparse to moderately dense sporulation, no exudate, no soluble pigment, reverse grayish green, and reached 20–24 mm in diameter. YES 25°C, 7 days: colonies were deep, raised at center, radially and

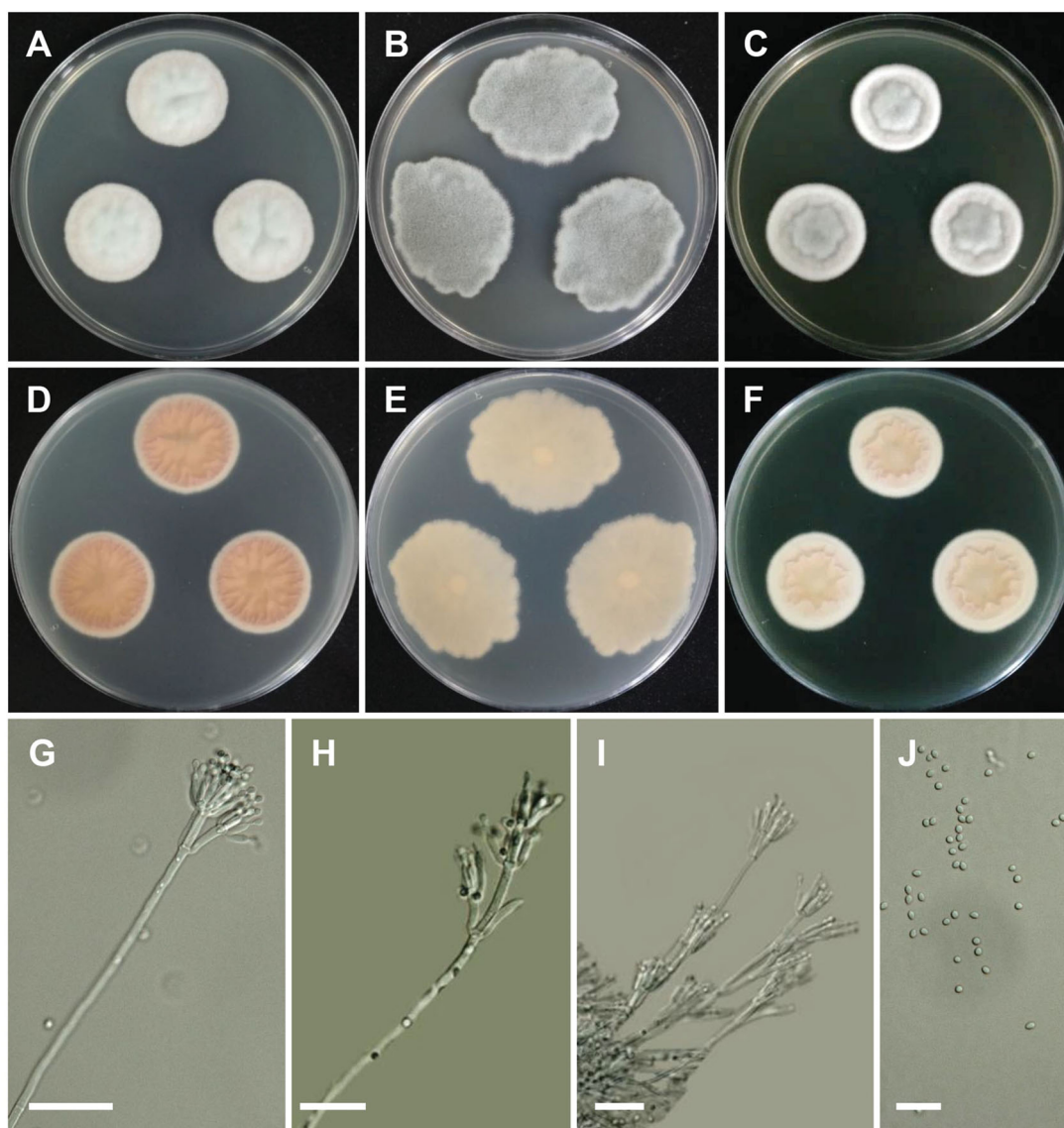
concentrically sulcate, low, narrow, entire margins, white mycelia, floccose, sulcate texture, sparse to moderately dense sporulation, no exudate, no soluble pigment, reverse grayish orange, and reached 16–19 mm in diameter.

Micromorphology: Conidiophores were mono- and biverticillate, 42–162 × 3.6–4 µm. Metulae were appressed to divergent, 7.6–20.2 × 2.7–4 µm. Phialides were ampulliform, 3–9 per metula, 7.8–12.6 × 2.2–4 µm. Conidia were globose to subglobose, 2.9–4.1 × 2.1–3.8 µm.

### 3.2.5. Taxonomy of CNUFC DMS3-17

*Penicillium limosum* S. Ueda, Mycoscience 36 (4): 451 (1995) (Figure 6).

Colony characteristics: CYA 25°C, 7 days: colonies were radially sulcate at center, with light grayish green to white floccose surface, no exudate and soluble pigment, reverse brown to brownish orange, and reached 39–44 mm in diameter. MEA 25°C,



**Figure 5.** Morphology of *Penicillium globosum*. (A,D) Colonies on Czapek yeast autolysate agar (CYA); (B,E) Colonies on Blakeslee's malt extract agar (MEA); (C,F) Colonies on yeast malt extract agar (YES). (A–C: obverse view, D–F: reverse view). (G–I) Conidiophores; (J) Conidia (scale bars: G–J = 20  $\mu$ m).

7 days: colonies were plain, white to grayish green mycelia, with sparse to moderately dense sporulation, no soluble pigment, reverse grayish yellow, and reached 35–41 mm in diameter. YES 25 °C, 7 days: colonies were deep, raised at center, radially and concentrically sulcate, with low, narrow, entire margins, white mycelia, floccose, sulcate texture, sparse to moderately dense sporulation, no exudate, no soluble pigment, reverse grayish orange, and reached 36–40 mm in diameter.

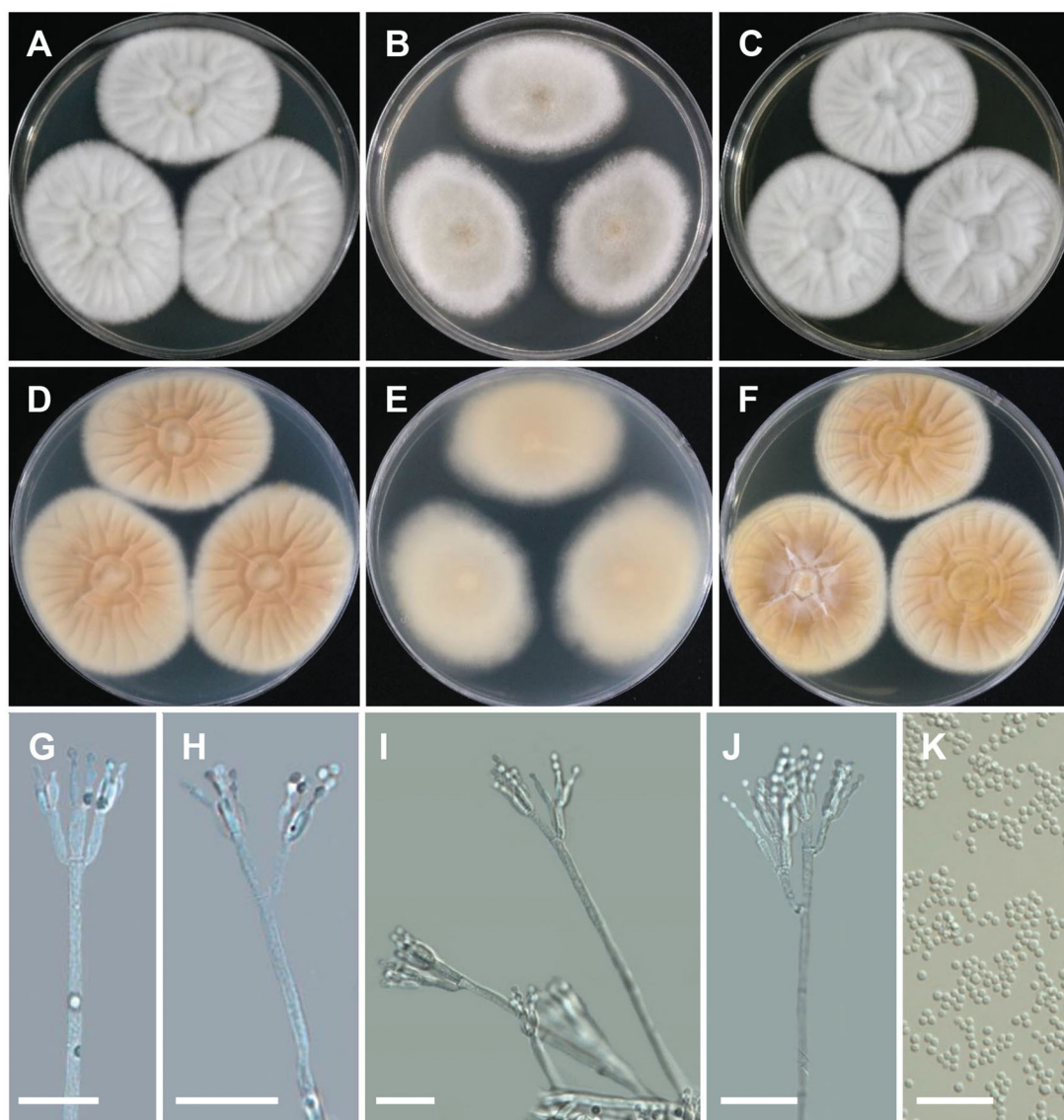
**Micromorphology:** Conidiophores were biverticillate, 73–252  $\mu$ m. Metulae were appressed to divergent, 14.8–20.2  $\times$  3–4  $\mu$ m. Phialides were ampulliform, 5–6 per metula, 8–12.6  $\times$  2.2–3  $\mu$ m. Conidia were globose to subglobose, 2.7–3.2  $\times$  2.4–3  $\mu$ m.

### 3.2.6. Taxonomy of CNUFC WG2-1

*Penicillium onobense* C. Ramírez & A.T. Martínez, Mycopathologia 74 (1): 44 (1981) (Figure 7).

**Colony characteristics:** CYA 25 °C, 7 days: colonies were velvety, radiately wrinkled, dirty white, entire margins, white mycelia, velutinous texture, moderately dense sporulation, no soluble pigments and exudates, reverse pale-brownish orange, and reached 19–22 mm in diameter. MEA 25 °C, 7 days: colonies were low, with low, irregular margins, green mycelia, grayish green at center, floccose texture, sparse to moderately dense sporulation, no exudate, no soluble pigment, reverse grayish green with light orange circle at center, and reached 36–43 mm in diameter. YES 25 °C, 7 days: colonies were moderately deep, radially and concentrically sulcate, with low, wide, entire margins, white mycelia, floccose texture, moderately dense sporulation, no soluble pigments, no exudates, reverse light-brown, and reached 18–23 mm in diameter.

**Micromorphology:** Conidiophores were biverticillate, 73–310  $\mu$ m. Metulae were divergent,



**Figure 6.** Morphology of *Penicillium limosum*. (A,D) Colonies on Czapek yeast autolysate agar (CYA); (B,E) Colonies on Blakeslee's malt extract agar (MEA); (C,F) Colonies on yeast malt extract agar (YES). (A–C: obverse view, D–F: reverse view). (G–J) Conidiophores; (K) Conidia (scale bars: G–K = 20  $\mu$ m).

10.2–13.1  $\times$  3.3–5  $\mu$ m. Phialides were ampulliform, 3–6 per metula, 8.3–12.2  $\times$  2.2–3  $\mu$ m. Conidia were elliptical to subglobose, 2.3–4.1  $\times$  2.1–4.1  $\mu$ m.

### 3.2.7. Taxonomy of *CNUFC WNP1-1*

*Penicillium yunnanense* L. Cai & X.Z. Jiang, Cladistics 35 (5): 545 (2018) (Figure 8).

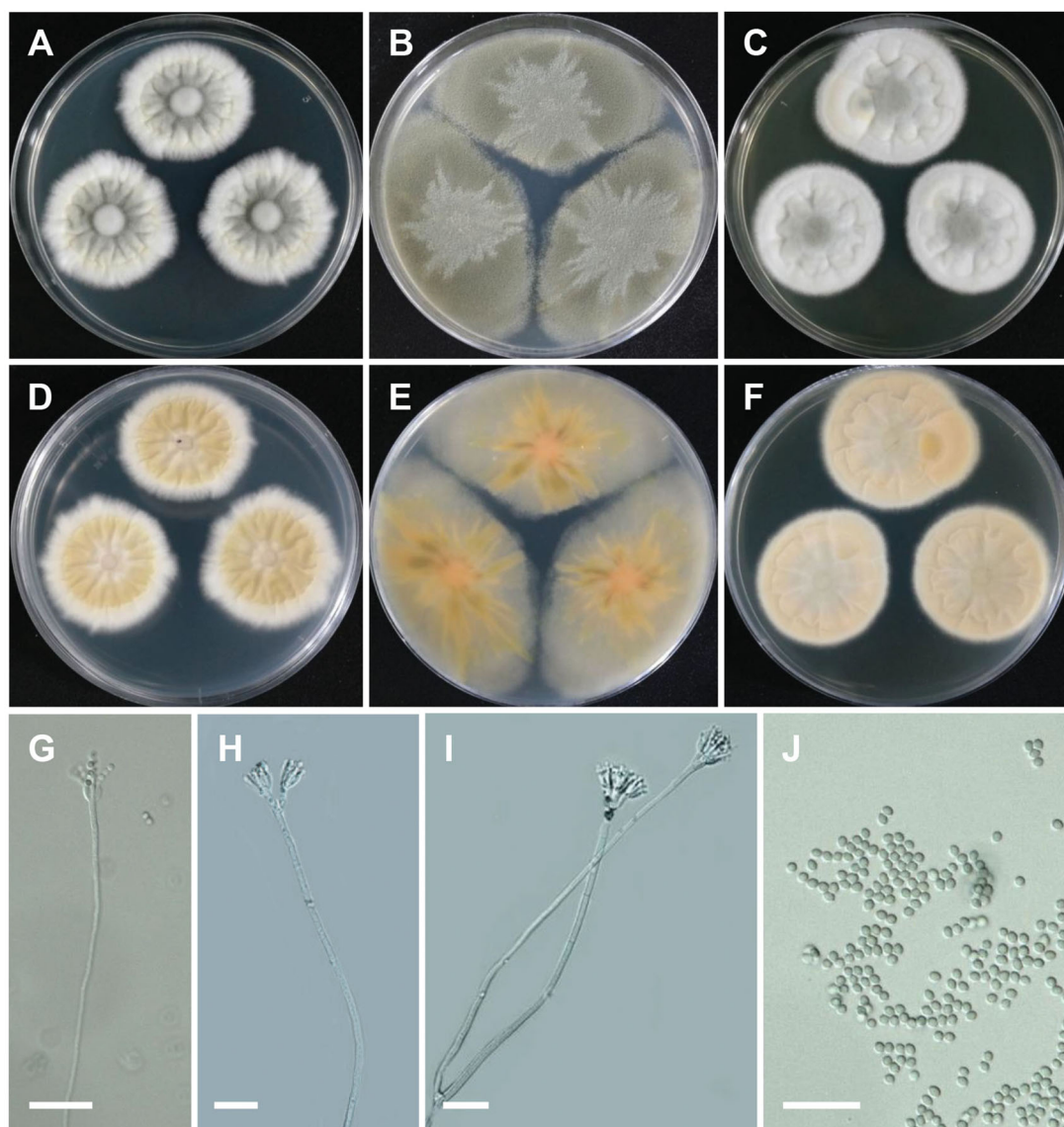
Colony characteristics: CYA 25 °C, 7 days: colonies were moderately deep, raised at center, radially sulcate, with low, narrow, entire margins, white mycelia, velutinous texture, moderately dense sporulation, no soluble pigments, no exudates, reverse greenish gray to dull-green, and reached 22–24 mm in diameter. MEA 25 °C, 7 days: colonies were moderately deep, radially sulcate, with low, narrow, irregular margins, white mycelia, floccose texture, sparse to moderately dense sporulation, no exudate, no soluble pigment, reverse pale to yellowish gray, pale yellowish circles at center, and reached

23–24 mm in diameter. YES 25 °C, 7 days: colonies were low to moderately deep, sunken at center, radially and concentrically sulcate, with low, narrow, entire margins, white mycelia, floccose texture, sparse to moderately dense sporulation, no exudate, no soluble pigment, reverse dull-green, and reached 26–28 mm in diameter.

Micromorphology: Conidiophores were mono- and biverticillate, 22–126  $\times$  2–3  $\mu$ m. No metulae were observed. Phialides were ampulliform, 6.6–14.1  $\times$  2–4  $\mu$ m. Conidia were broadly ellipsoidal to ellipsoidal, 3–4  $\times$  2.5–3.5  $\mu$ m.

## 4. Discussion

This study advanced our understanding of *Penicillium* sect. *Lanata-Divaricata* and contributed seven new records for Korea.

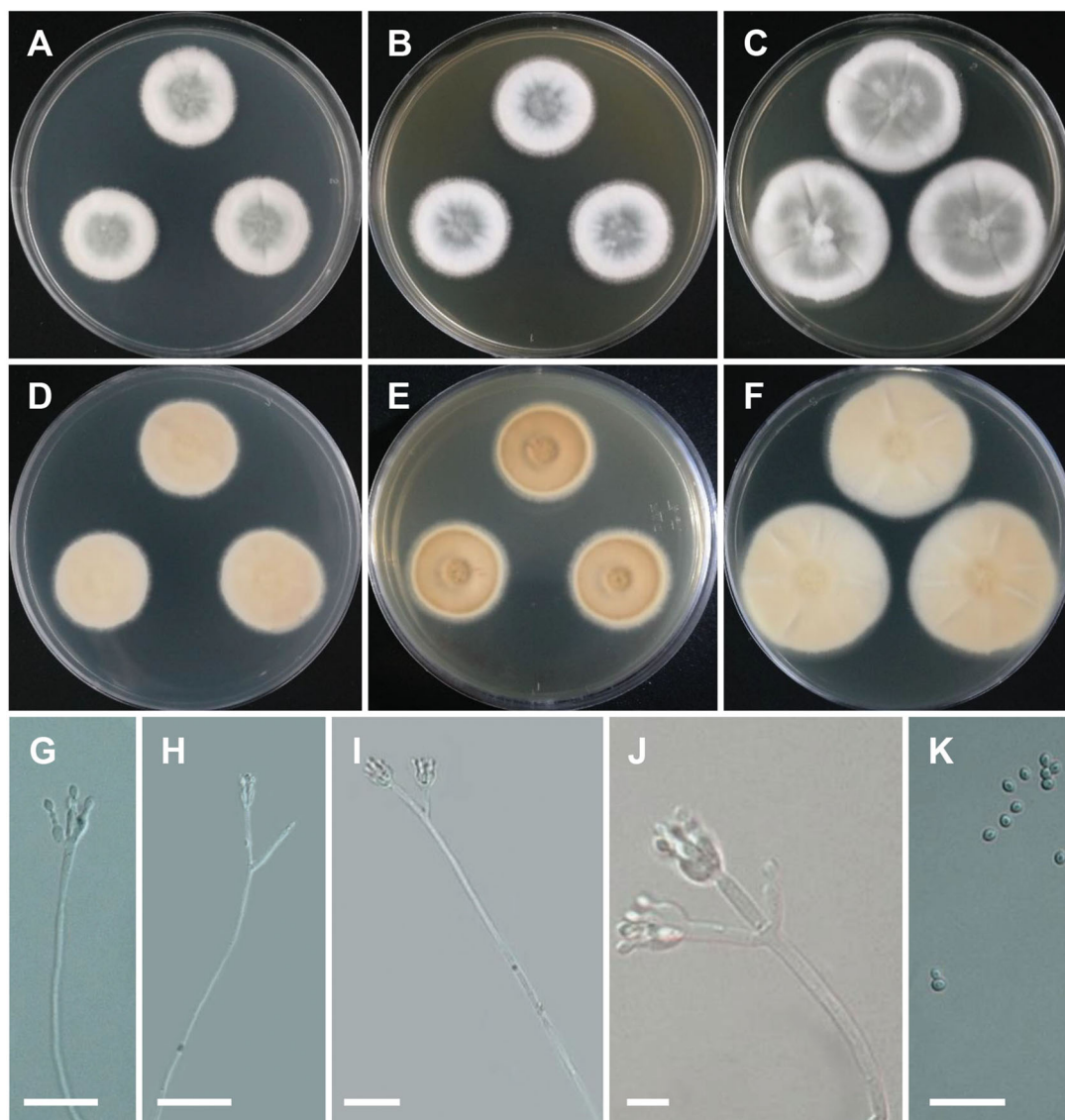


**Figure 7.** Morphology of *Penicillium onobense*. (A,D) Colonies on Czapek yeast autolysate agar (CYA); (B,E) Colonies on Blakeslee's malt extract agar (MEA); (C,F) Colonies on yeast malt extract agar (YES). (A–C: obverse view, D–F: reverse view). (G–I) Conidiophores; (J) Conidia (scale bars: G–J = 20 μm).

Although ITS rDNA is designated as the universal primers available and most widely sequenced marker for fungi [30], but not variable enough for distinguishing all closely related species in *Penicillium* [3,31]. Thus, additional secondary marker such as *BenA* and *CaM* were proposed and found to be useful for the accurate identification of *Penicillium* species [2,3,15]. Phylogenetic tree based on combined ITS-*BenA*-*CaM* sequences indicated that seven strains belonging to the series *Janthinella*, *Rolfsiorum* and *Simplicissima* in section *Lanata-Divaricata* (Figure 1).

The isolates CNUFC ULD4-13 was well placed within the clade of *P. annulatum* in series *Rolfsiorum* (Figure 1). The isolate was morphologically similar to description of *P. annulatum* [15] with respect to producing bi- and terverticillate conidiophores, ampulliform phialides, and roughened

globose to subglobose conidia (Figure 2). Previous studies showed *P. annulatum* to be isolated from air sample, soil, and mite in *Protea repens* infructescence and Stellenbosch in South Africa [15]. There are no studies on extrolites produced by *P. annulatum*. Similarly, the phylogenetic placement and morphological characteristics of isolates CNUFC ULD4-3 into series *Rolfsiorum* (Figure 1), matched previously with described species, *P. camponotum* [16]. *Penicillium camponotum* isolated from carpenter ants from New Brunswick, Canada, and ant nest in *Picea abies* from Germany, was reported to produce andrastin A, B, and C, citrinalin, mangrovamides, marcfortine A and B, and patulin [16]. In this study, *P. camponotum* was isolated from soil samples. The isolate CNUFC MDW-11 was grouped with *P. echinulonalgiovense* in series *Simplicissima* (Figure 1) and shared morphology similar to the previous



**Figure 8.** Morphology of *Penicillium yunnanense*. (A,D) Colonies on Czapek yeast autolysate agar (CYA); (B,E) Colonies on Blakeslee's malt extract agar (MEA); (C,F) Colonies on yeast malt extract agar (YES). (A–C: obverse view, D–F: reverse view). (G–J) Conidiophores; (K) Conidia (scale bars: G–K = 20 μm).

descriptions. *Penicillium echinulonalgiovense* was first isolated from soil samples in Japan without a Latin diagnosis, later validated by Barbosa et al. [32,33]. *Penicillium echinulonalgiovense* was also isolated from bee pollen and nest of *Melipona scutellaris* located at Recife, Pernambuco in Brazil; soil samples from Australia, China, Hong Kong, Indonesia, USA, Madagascar, and Malaysia; and industrial installations in Netherlands [33]. *Penicillium echinulonalgiovense* produces andrastin A, xanthoepocin, and pulvilloric acid [2,33]. Similarly, CNUFC WG2-7 was clustered within the same clade as *P. globosum* in series *Simplicissima* (Figure 1), described by Diao et al. [34]. The isolate CNUFC WG2-7 was morphologically similar to *P. globosum* with only differences in the number of phialides per metula. According to Diao et al. [34], 3–19 phialides per metula whereas the isolate CNUFC WG2-7 consists of 3–9 phialides per

metula. *P. globosum* was isolated from an acidic soil in China and Australia; rainforest soil from Malaysia; industrial installations in Netherlands; and soil in citrus grove in Florida, USA [34]. Also, the morphological characteristics of CNUFC DMS3-17 were similar to the previous descriptions [35] and was also clustered in *P. limosum* CBS 339.97 (type) in series *Janthinella* (Figure 1). Earlier, *Penicillium limosum* have been reported to be found only in marine sediment in Nagasaki Prefecture, Japan [35]. In this study, we isolated *P. limosum* from damp soil samples. *Penicillium limosum* was reported to produce a sexual state [35] but no sexual state is found in CNUFC DMS3-17 isolate. Based on previous descriptions, CNUFC WG2-1 shared similar morphology and placed in series *Simplicissima* (Figure 1). *Penicillium onobense* isolated from soil and andosol in Navarra, Spain [36], produces brefeldin A, janthitrems/shearinins, and 2-(4-

hydroxyphenyl)-2-oxoacetaldehyde oxime [2]. The isolate CNUFC WNP1 was placed in series *Janthinella* (Figure 1) and also shared similar morphological characters with previous descriptions [34]. *P. yunnanense* was isolated from acidic soil in China [34]. Interestingly, this study isolated *P. echinulonalgiovense*, *P. globosum*, *P. onobense*, and *P. yunnanense* from freshwater for the first time.

This study on the isolation and description of seven new records of *Penicillium* sect. *Lanata-Dirivata* from freshwater and soil samples adds to our knowledge on fungal biodiversity. Different environmental sources such as bees, ants, flowers, leaves, nut kernels, and shells studies are needed in Korea considering the increasing evidence for ecological specialization in *Penicillium* species. Additional studies on the production of extracellular enzymes, antimicrobial compounds, and extrolites are still needed in the genus *Penicillium*.

### Disclosure statement

No potential conflict of interest was reported by the author(s).

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